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Epoxyeicosatrienoic acids, potassium channel blockers and endothelium-dependent hyperpolarization in the guinea-pig carotid artery

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1 Using intracellular microelectrodes, we investigated the effects of 17-octadecynoic acid (17-ODYA) on the endothelium-dependent hyperpolarization induced by acetylcholine in the guinea-pig isolated internal carotid artery with endothelium.

2 In the presence of N^{ω}-nitro-L-arginine (L-NOARG, 100 μM) and indomethacin (5 μM) to inhibit nitric oxide synthase and cyclo-oxygenase, acetylcholine (1 μM) evoked an endothelium-dependent hyperpolarization which averaged -16.4 mV starting from a resting membrane potential of -56.8 mV. There was a negative correlation between the amplitude of the hyperpolarization and the absolute values of the resting membrane potential.

3 The acetylcholine-induced endothelium-dependent hyperpolarization was not altered by charybdotoxin (0.1 μM) or iberiotoxin (30 nM). It was partially but significantly reduced by apamin (0.5 μM) to -12.8 ± 1.2 mV ($n=10$) or the combination of apamin plus iberiotoxin (-14.3 ± 3.4 mV, $n=4$). However, the combination of charybdotoxin and apamin abolished the hyperpolarization and under these conditions, acetylcholine evoked a depolarization ($+7.1 \pm 3.7$ mV, $n=8$).

4 17-ODYA (10 μM) produced a significant hyperpolarization of the resting membrane potential which averaged -59.6 mV and a partial but significant inhibition of the acetylcholine-induced endothelium-dependent hyperpolarization (-10.9 mV).

5 Apamin did not modify the effects of 17-ODYA but in the presence of charybdotoxin or iberiotoxin, 17-ODYA no longer influenced the resting membrane potential or the acetylcholine-induced hyperpolarization.

6 When compared to solvent (ethanol, 1% v/v), epoxyeicosatrienoic acids (EpETrEs) (5,6-, 8,9-, 11,12- and 14,15-EpETrE, 3 μM) did not affect the cell membrane potential and did not relax the guinea-pig isolated internal carotid artery.

7 These results indicate that, in the guinea-pig internal carotid artery, the involvement of metabolites of arachidonic acid through the cytochrome P_{450} pathway in endothelium-dependent hyperpolarization is unlikely. Furthermore, the hyperpolarization mediated by the endothelium-derived hyperpolarizing factor (EDHF) is probably not due to the opening of BK_{Ca} channels.

Keywords: Acetylcholine; cytochrome P_{450}; EDHF; endothelium; epoxyeicosatrienoic acids; hyperpolarization; iberiotoxin; smooth muscle; 17-ODYA

Introduction

Endothelial cells release various vasoactive substances such as NO (Furchgott & Zawadzki, 1980; Palmer *et al*., 1987), prostacyclin (Moncada & Vane, 1979) and a yet unidentified endothelium-derived hyperpolarizing factor (EDHF) which causes membrane hyperpolarization by opening K^+ channels in vascular smooth muscle cells (Félétou & Vanhoutte, 1988; Chen *et al*., 1988; 1991; Corriu *et al*., 1996b). EDHF could be a labile metabolite of arachidonic acid produced by the cytochrome P_{450} mono-oxygenase pathway, in particular an epoxyeicosatrienoic acid which opens calcium activated potassium channels of large conductance (BK_{Ca} channels) (Komori & Vanhoutte, 1990; Campbell *et al*., 1996; Chen & Cheung, 1996; Fang *et al*., 1996; Popp *et al*., 1996). However, in arteries from different species, cytochrome P_{450} inhibitors either do not affect endothelium-dependent hyperpolarization or produce an inhibition of L-NOARG-resistant endothelium-dependent relaxation and/or hyperpolarization only at concentrations which are no longer specific for P_{450} mono-oxygenases (Corriu *et al*., 1996a; Zygmunt *et al*., 1996; Graier *et al*., 1996a,b).

In guinea-pig carotid and rat hepatic arteries, endothelium-dependent hyperpolarizations and relaxations (resistant to inhibitors of nitric oxide synthase and cyclo-oxygenase) are abolished by the combination of two potassium channel blockers, charybdotoxin (BK_{Ca} channel blocker) and apamin (blocker of calcium activated potassium channels of small conductance, SK_{Ca} channel blocker), whereas each blocker by itself is ineffective (Corriu *et al*., 1996b; Garland & Plane, 1996; Zygmunt & Högestätt, 1996). These observations would be explained if two different EDHFs released by the endothelial cells activate two different populations of potassium channels, one sensitive to apamin and the other to charybdotoxin. A similar pattern is observed, for example, in the non-adrenergic, non-cholinergic response of gastrointestinal smooth muscle (Kishi *et al*., 1996; Maggi & Giuliani, 1996). Thus, in some arteries cytochrome P_{450} metabolites of arachidonic acid which activate BK_{Ca} channels, could be only one of the EDHF(s) released by endothelial cells. In blood vessels such as the guinea-pig carotid artery, the inhibition of cytochrome P_{450}

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would produce a similar effect as charybdotoxin alone, i.e. no apparent inhibition of the endothelium-dependent hyperpolarization. The aim of the present experiments was to test this hypothesis by measuring the changes in smooth muscle cell membrane potential in the guinea-pig carotid artery treated with a cytochrome P_{450} mono-oxygenase inhibitor, in the absence or presence of various potassium channel blockers.

Methods

Electrophysiological experiments

Male Hartley guinea-pigs (250–350 g) were anaesthetized by intraperitoneal administration of pentobarbitone (300 mg kg^{-1}) and the carotid arteries with their branches were dissected free. Segments of artery (1 cm in length) were cleaned of adherent connective tissues and pinned down to the bottom of an organ chamber (0.5 ml in volume) superfused at a constant flow (2 ml min^{-1}) with modified Krebs-Ringer bicarbonate solution (37°C, aerated with a 95% O_2/5% CO_2 gas mixture, pH 7.4). This solution was of the following composition (in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, calcium-disodium EDTA 0.026 and glucose 11.1. In most experiments, care was taken to preserve the endothelium as intact as possible; in some experiments, the endothelium was destroyed by a rapid infusion of saponin (1 mg ml^{-1}) in the lumen of the blood vessel (Corriu *et al*., 1996b). Endothelial cell removal was considered to be successful when the hyperpolarization induced by acetylcholine (1 μM) did not exceed 5 mV (average: -1.0 ± 0.6, $n=10$). Transmembrane potentials were recorded from the adventitial side of the internal carotid arteries with glass capillary microelectrodes (tip resistance of 30 to 90 MΩ) filled with KCl (3 M) and connected to the headstage of a recording amplifier (World Precision Instruments (intra 767), New Haven, CT) with capacitance neutralization; an Ag/AgCl pellet, in contact with the bathing solution and directly connected to the amplifier, served as the reference electrode. The signal was continuously monitored on an oscilloscope (3091 Nicolet, Madison, WI) and simultaneously recorded on paper (Gould, Valley View, OH) and on a video recorder (TEAC XR310; Tokyo, Japan). Successful impalements were signalled by a sudden negative drop in potential from the baseline (zero potential reference) followed by a stable negative potential for at least 3 min. The incubation time was at least 30 min with the various potassium channel inhibitors studied, and 75 min with the cytochrome P_{450} mono-oxygenase inhibitor, 17-octadecynoic acid (17-ODYA). Acetylcholine was infused for no longer than 5 min to avoid desensitization of the preparation. All the experiments were performed in the presence of L-NOARG (100 μM) and indomethacin (5 μM) in order to inhibit nitric oxide synthase and cyclo-oxygenase, respectively.

Myograph experiments

Segments of internal carotid arteries were mounted in myographs (Mulvany & Halpern, 1977) and threaded onto two polyamide wires (20 μm in diameter). The wires were attached to a support and the contraction was recorded. Segments were stretched step by step (passive tension approximately 250 mg) and contracted with KCl (60 mM) until reproducible contraction to the depolarizing solution was obtained (232 ± 26 mg, $n=11$). After a 45 min equilibration period, the internal carotid arteries were contracted with the thromboxane A_2 analogue U 46119 (9,11-dideoxy-9α,11α-epoxymethanoprostaglandin $F_{2\alpha}$ 100 nM), and then treated with L-NOARG (100 μM) plus indomethacin (5 μM) when the contraction was stabilised. EpETrE (3 μM) were tested 40 min later, then acetylcholine (1 μM) was applied and finally sodium nitroprusside (1 mM) was added. Changes in tension are expressed as % of the active tension which was indicated by the maximal relaxation produced by sodium nitroprusside (1 mM).

Drugs

The drugs used were: acetylcholine chloride, indomethacin, N^{ω}-nitro-L-arginine (L-NOARG), 17-octadecynoic acid (17-ODYA), sodium nitroprusside (Sigma, La Verpillère, France); charybdotoxin, apamin and iberiotoxin (Latoxan, Rosans, France); (8Z, 11Z, 14Z)-racemic-*cis*-5(6)-epoxyeicosatrienoic acid (5,6-EpETrE), (5Z, 11Z, 14Z)-racemic-*cis*-8(9)-epoxyeicosatrienoic acid (8,9-EpETrE), (5Z, 8Z, 14Z)-racemic-*cis*-11(12)-epoxyeicosatrienoic acid (11,12-EpETrE), (5Z, 8Z, 11Z)-racemic-*cis*-14(15)-epoxyeicosatrienoic acid (14,15-EpETrE) (Cascade Biochem Ltd, Reading, U.K.); cromakalim was synthesized in the Servier Research Center (Suresnes, France). Indomethacin was dissolved in deionized water and an equimolar concentration of Na_2CO_3. 17-ODYA and EpETrE(s) were dissolved in ethanol. Cromakalim was dissolved in equivalent volumes of deionized water and propylene glycol. The other drugs were dissolved in deionized water.

Statistics

Data are shown as mean ± s.e.mean; n indicates the number of cells in which membrane potential was recorded. Statistical analysis was performed by use of Student's t test for paired or unpaired observations. Differences were considered to be statistically significant when the P value was less than 0.05.

Results

Under the experimental conditions imposed, the resting membrane potential of the smooth muscle cells of the guinea-pig internal carotid artery with endothelium averaged -56.8 ± 0.8 mV ($n=62$). Acetylcholine (1 μM) induced an endothelium-dependent hyperpolarization which averaged -16.4 ± 0.6 mV ($n=62$). The amplitude of the hyperpolarizations induced by acetylcholine was correlated negatively with the absolute values of the resting membrane potential (Figure 1).

Potassium channel blockers

When compared to their respective controls, at the concentration tested, the potassium channel blockers, alone or in combination, did not induce a statistically significant change in resting membrane potential (control: -60.2 ± 1.7 mV, $n=5$ and charybdotoxin (0.1 μM): -51.0 ± 5.9 mV, $n=5$; control: -57.8 ± 1.4 mV, $n=5$ and iberiotoxin (30 nM): -55.3 ± 3.2 mV, $n=6$; control: $-55.2 + 1.2$ mV, $n=9$ and apamin (0.5 μM): -59.5 ± 2.4 mV, $n=10$; control: $-56.0 + 1.1$ mV, $n=4$ and apamin plus iberiotoxin: -60.3 ± 4.1 mV, $n=4$; control: 58.2 ± 1.7 mV, $n=6$ and apamin plus charybdotoxin: -54.8 ± 4.1 mV, $n=8$).

The endothelium-dependent hyperpolarization obtained in response to acetylcholine (1 μM) was not significantly affected by charybdotoxin or iberiotoxin. Apamin significantly reduced

the hyperpolarization induced by acetylcholine. The combination of iberiotoxin and apamin did not induce a greater inhibition than apamin alone. The combination of charybdotoxin plus apamin abolished the hyperpolarization to acetylcholine which instead produced a significant depolarization (Figure 2).

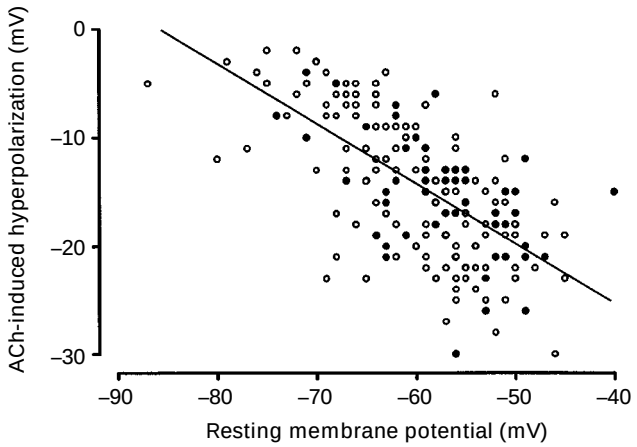

Figure 1 Relationship between the amplitude of acetylcholine (ACh, 1 μM)-induced endothelium-dependent hyperpolarizations and resting membrane potential in the guinea-pig carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). A significant correlation was observed ($r=0.66$, $n=196$). The individual values shown represent findings from the present study (solid circles) as well as data obtained in previous studies (open circles) (Corriu *et al*., 1996a,b, by permission).

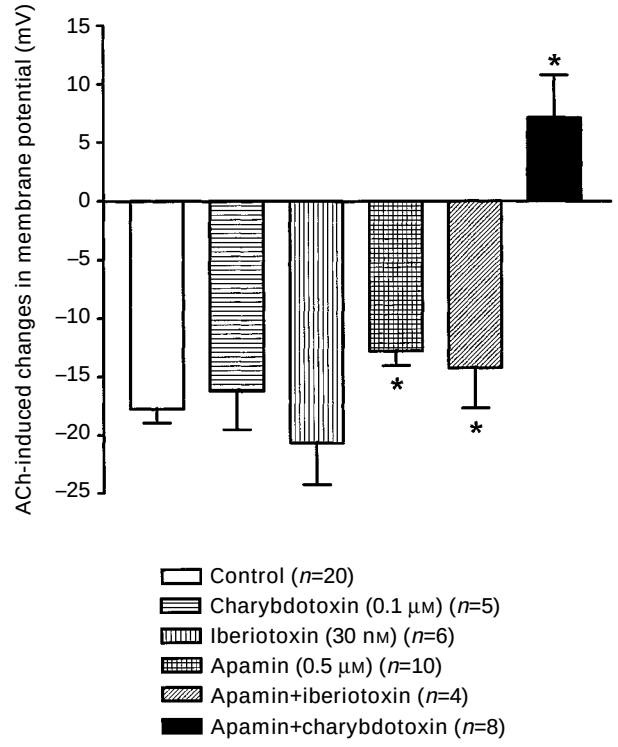

Figure 2 Effect of potassium channel blockers on the endothelium-dependent hyperpolarization evoked by ACh (1 μM) in the guinea-pig internal carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean$\pm$s.e.mean. For the sake of clarity, the control values have been pooled. The asterisk indicates a statistical difference versus the respective control values with $P<0.05$.

Cytochrome P_{450} mono-oxygenase inhibitor

When compared to their respective controls, at 5 μM, 17-ODYA did not influence significantly the resting membrane potential (control: -52.0 ± 1.0, $n=2$ and 17-ODYA: 53.2 ± 2.5 mV, $n=5$). At 10 μM, the cell membrane was significantly hyperpolarized (control: -55.0 ± 2.0, $n=11$ and 17-ODYA: -59.6 ± 2.3 mV, $n=13$). The endothelium-dependent hyperpolarization in response to acetylcholine (1 μM) was not affected significantly by the lower, but partially and significantly inhibited by the higher concentration of 17-ODYA tested (Figure 3).

Cytochrome P_{450} mono-oxygenase inhibitor in combination with potassium channel blockers

The combinations of 17-ODYA (10 μM) plus charybdotoxin (0.1 μM) and 17-ODYA (10 μM) plus iberiotoxin (30 nM) did not influence significantly the resting membrane potential (control: -57.3 ± 2.6 mV; $n=4$, and 17-ODYA + charybdotoxin: -54.4 ± 5.9 mV, $n=5$; control: 56.3 ± 2.5 mV, $n=4$ and 17-ODYA + iberiotoxin: -56.7 ± 3.6 mV, $n=6$). The combination of 17-ODYA (10 μM) plus apamin (0.5 μM) significantly hyperpolarized the cell membrane (control: -53.8 ± 2.0, $n=11$ and 17-ODYA + apamin: -59.1 ± 2.8 mV, $n=11$).

In the presence of the combination of 17-ODYA plus apamin, the endothelium-dependent hyperpolarization was inhibited significantly when compared to the control, but was not significantly different from the hyperpolarizations observed in the presence of either apamin or 17-ODYA alone. The combination of 17-ODYA (10 μM) plus charybdotoxin (0.1 μM) or that of 17-ODYA (10 μM) plus iberiotoxin (30 nM) did not significantly affect the endothelium-dependent hyperpolarization evoked by acetylcholine (Figure 4).

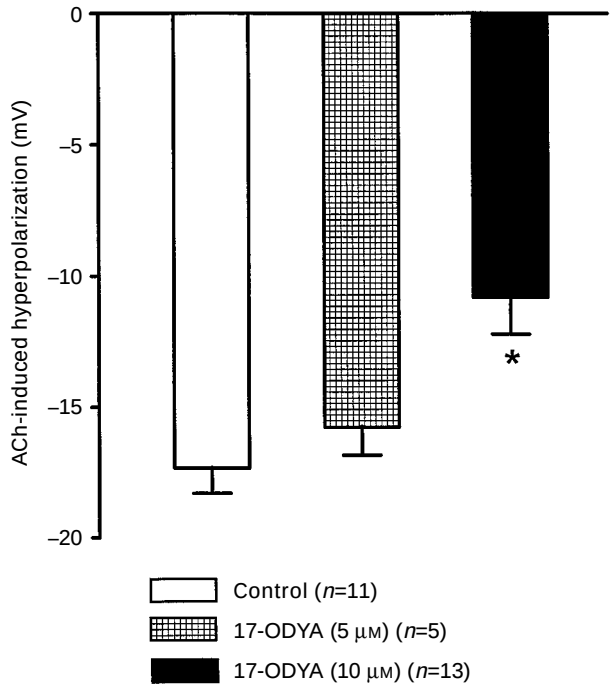

Figure 3 Effect of the cytochrome P_{450} mono-oxygenase inhibitor, 17-ODYA, on the endothelium-dependent hyperpolarization evoked by ACh (1 μM) in guinea-pig internal carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean$\pm$s.e.mean. For the sake of clarity, the control values have been pooled. The asterisk indicates a statistically significant difference versus the respective control values with $P<0.05$.

In vessels without endothelium, the membrane potential was -51.1 ± 1.6 ($n=10$) in control conditions and -54.1 ± 2.0 ($n=10$) in the presence of 17-ODYA (10 μM). Although this difference is not statistically significant, in 8 out of 10 tissues a more negative potential was observed after 17-ODYA treatment. In the presence of iberiotoxin (30 nM) and 17-ODYA (10 μM) the membrane potential was -46.3 ± 1.9 ($n=4$), which was not significantly different from control but significantly different from that in the presence of 17-ODYA alone.

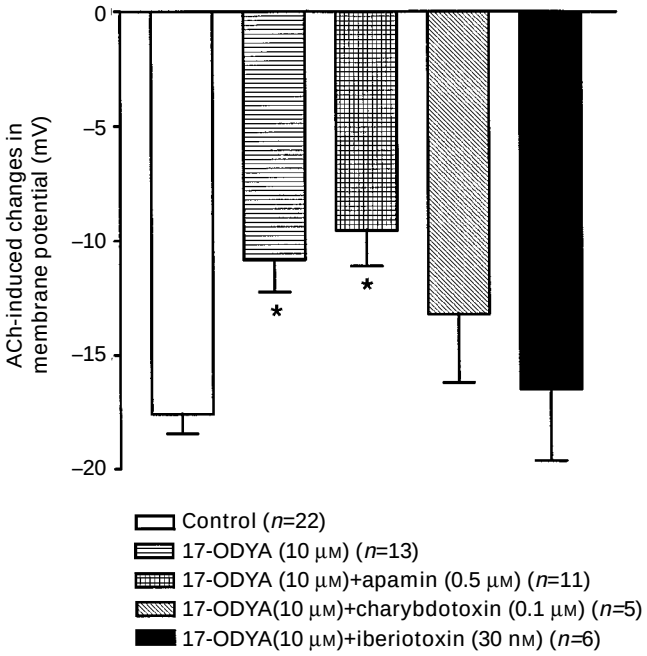

Figure 4 Effect of the combination of 17-ODYA (10 μM) plus potassium channel blockers on the endothelium-dependent hyperpolarization produced by ACh (1 μM) in guinea-pig internal carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean $\pm$ s.e.mean. For the sake of clarity, the control values have been pooled. The asterisk indicates a statistically significant difference versus the respective control values with $P<0.05$.

Cromakalim

The hyperpolarizations produced by cromakalim (1 and 10 μM: -18.0 ± 0.6 mV, $n=3$ and -22.0 ± 2.9 mV, $n=6$, respectively) were not affected significantly by 17-ODYA (10 μM) (-16.7 ± 1.7 mV, $n=3$ and -17.9 ± 1.9 mV, $n=7$, respectively).

EpETrE(s)

After a successful impalement and stabilization of the membrane potential, the superfusion of the preparation was stopped. The EpETrE(s) was retrieved from cold storage (-20°C or -70°C), and 5 μl were directly removed from the vials and injected near the preparation (dilution 1% v/v,

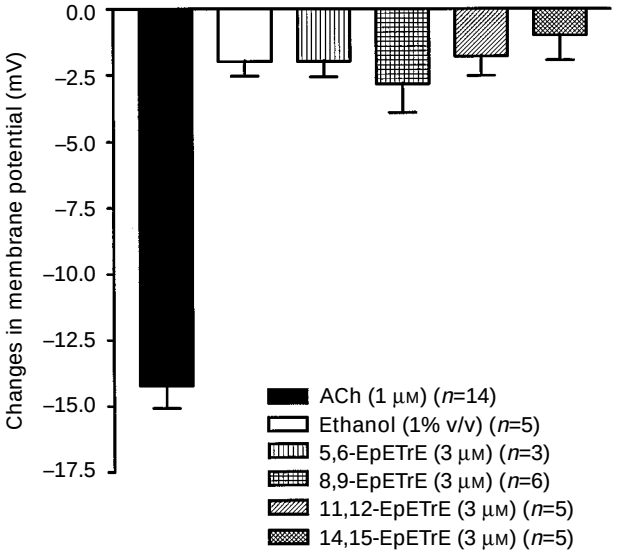

Figure 5 Effects of ACh and epoxyeicosatrienoic acids (EpETrEs: 3 μM) in guinea-pig internal carotid artery in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean $\pm$ s.e.mean.

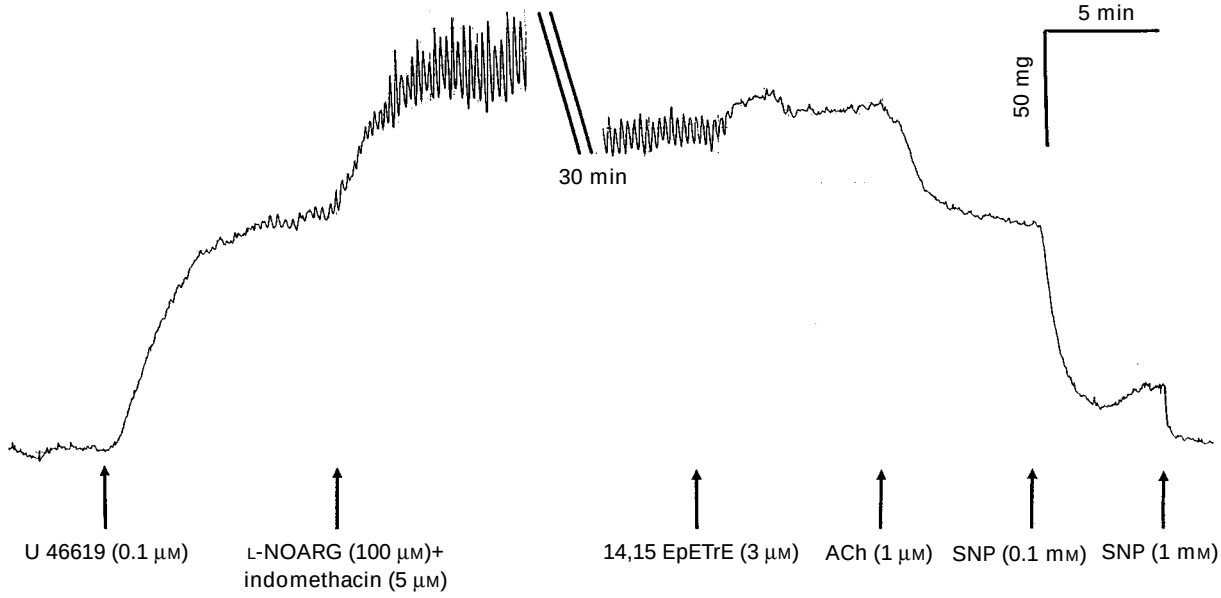

Figure 6 Original traces showing the changes of tension produced by 14,15 EpETrE (3 μM), acetylcholine (ACh: 1 μM) and sodium nitroprusside (SNP: 0.1 and 1 mM) in the guinea-pig isolated internal carotid artery with endothelium contracted with U 46619 (0.1 μM) in the presence of N^{ω}-nitro-L-arginine (L-NOARG: 100 μM) and indomethacin (5 μM).

approximate final concentration 3 μM) in order to minimize a potential rapid degradation of the EpETrE(s) in the oxygenated Krebs-Ringer bicarbonate solution. When compared to the solvent (ethanol, 1% v/v), 5,6-EpETrE, 8,9-EpETrE, 11,12-EpETrE and 14,15-EpETrE did not induce statistically significant hyperpolarization. Under identical experimental conditions, acetylcholine (1 μM) applied in the presence or absence of the EpETrEs induced a hyperpolarization which averaged -14.2 ± 0.9 mV ($n=14$) (Figure 5).

Myograph experiments

Isolated internal carotid arteries were contracted with U46619 (100 nM). The tension averaged 68 ± 12 mg (29 ± 4% of KCl-induced contraction; $n=11$). Addition of the combination of L-NOARG (100 μM) plus indomethacin (5 μM) increased the tension to 159 ± 19 mg (69 ± 4% of KCl-induced contraction; $n=11$). None of the EpETrEs tested (5,6-EpETrE, 8,9-EpETrE, 11,12-EpETrE and 14,15-EpETrE: 3 μM) induced any significant relaxation. However, the addition of acetylcholine (1 μM) produced consistent and significant relaxations (Figures 6 and 7).

Discussion

The present study indicates that metabolites of arachidonic acid formed by the cytochrome P_{450} mono-oxygenase pathway are unlikely to play a major role in the endothelium-dependent hyperpolarization to acetylcholine (resistant to the blockade of both nitric oxide synthase and cyclo-oxygenase) in the isolated internal carotid artery of the guinea-pig.

The findings confirmed that in the guinea-pig carotid artery, the endothelium-dependent hyperpolarization attributed to EDHF is abolished by the combination of both charybdotoxin and apamin (Corriu *et al*., 1996b). We have previously demonstrated that this inhibition is only observed in the presence of inhibitors of NO-synthase and cyclo-oxygenase. In the absence of these enzyme inhibitors, the combination of charybdotoxin plus apamin does not block the hyperpolarization due to the release of endothelial NO and/or prostacyclin (Corriu *et al*., 1996b). Similar observations have been presented by Zygmunt and Högestätt (1996) and Petersson *et al*. (1997) studying the endothelium-dependent relaxations of rat mesenteric and guinea-pig cerebral arteries, respectively. This strongly suggests that the site of action of these two toxins is more likely to be the smooth muscle cells than the endothelium. However, the possibility cannot be excluded that these toxins interfere with the acetylcholine effects on the endothelial cells thus resulting in changes in EDHF production. Only bioassay experiments allowing selective treatment of either endothelial or smooth muscle cells will definitively answer the question. The combination of iberiotoxin plus apamin did not mimic the effects of charybdotoxin and apamin. A similar finding has been demonstrated in the rat hepatic artery for the relaxation resistant to L-NOARG (Zygmunt & Högestätt, 1996). As iberiotoxin is supposed to be more selective towards BK_{Ca} channels than charybdotoxin (Candia *et al*., 1992), these results do not support the involvement of BK_{Ca} channels in the endothelium-dependent hyperpolarization of the guinea-pig internal carotid artery. In contrast, a previous study showed that scillatoxin, a SK_{Ca} channel blocker which is structurally different from apamin, mimicked the effect of apamin. Indeed, charybdotoxin plus scillatoxin abolished the endothelium-dependent hyperpolarization of the guinea-pig internal carotid artery in response to acetylcholine (Corriu *et al*., 1996b). In the rabbit mesenteric artery, apamin alone fully blocks endothelium-dependent hyperpolarization (Murphy & Brayden, 1995). In the present study, apamin alone produced a significant but partial inhibition of the hyperpolarization. There is no immediate explanation for this discrepancy with the previous study in which neither apamin nor scillatoxin significantly influenced the hyperpolarization (Corriu *et al*., 1996b). These results indicate that the potassium channel(s) involved in the endothelium-dependent hyperpolarization of the guinea-pig internal carotid artery do not correspond to an identified subtype and that the participation of SK_{Ca} channels or potassium channels containing subunit(s) of a SK_{Ca} channels is likely.

17-ODYA is a suicide substrate which selectively and irreversibly inhibits cytochrome P_{450} epoxygenases and ω-hydroxylases involved in the formation of EpETrEs and hydroxyeicosatrienoic acids (Zou *et al*., 1994). The present experiments confirm that in the guinea-pig internal carotid artery, 17-ODYA up to 5 μM does not influence the endothelium-dependent hyperpolarization to acetylcholine (Corriu *et al*., 1996a). However, by increasing the concentration to 10 μM, a partial but significant effect of this compound was observed both on the resting membrane potential and on the hyperpolarizing response evoked by acetylcholine. This inhibitory effect was not potentiated by apamin indicating that 17-ODYA does not suppress the charybdotoxin-sensitive component of this hyperpolarization. Therefore, this ruled out our initial hypothesis which considered the possibility of two different EDHF(s), one unknown factor activating apamin-sensitive potassium channels and an EpETrE activating charybdotoxin-sensitive potassium channels. Furthermore, the inhibition produced by 17-ODYA was reversed by charybdotoxin and by iberiotoxin suggesting that the effect of 17-ODYA is not linked to the suppression of one putative EDHF. The effects of 17-ODYA could be explained by a specific action of the compound. Indeed, in contrast to other cytochrome P_{450} inhibitors, such as proadifen or clotrimazole which also can block potassium and calcium channels, no

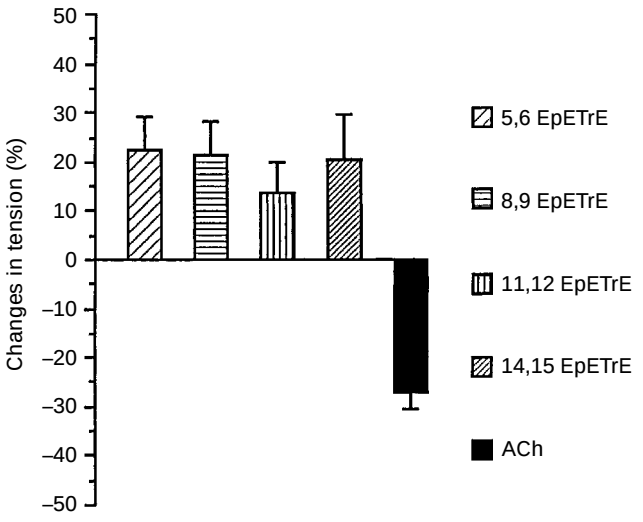

Figure 7 Changes in tension produced by epoxyeicosatrienoic acids (EpETrEs: 3 μM) and acetylcholine (ACh: 1 μM) in guinea-pig internal carotid artery contracted with U 46619 (0.1 μM) in the presence of L-NOARG (100 μM) and indomethacin (5 μM). Data are shown as mean $\pm$ s.e.mean. 5,6 EpETrE and 11,12 EpETrE: $n=5$; 8,9EpETrE and 14,15 EpETrE: $n=6$; ACh: $n=22$.

major non-specific effects have been attributed to 17-ODYA (Clementi & Meldolesi, 1996; Zygmunt *et al*., 1996; Edwards *et al*., 1996). In the present study, 17-ODYA did not affect the hyperpolarization induced by cromakalim, indicating that the cytochrome P_{450} inhibitor did not influence ATP-dependent potassium channels. 17-ODYA also cannot be regarded as an antagonist of the muscarinic receptor, since it causes only a minimal rightward shift in the concentration-contraction curve in the guinea-pig isolated tracheae in response to acetylcholine (unpublished observations). 17-ODYA hyperpolarized resting membranes of smooth muscle cells of the guinea-pig internal carotid artery. This hyperpolarization was no longer observed in the presence of charybdotoxin or iberiotoxin but still present in the presence of apamin. In vessels without endothelium the same trend was observed, e.g. an increase in membrane potential which was prevented by iberiotoxin. These results suggest that 17-ODYA opens BK_{Ca} channels, a phenomenon also described in freshly isolated single cells derived from the rat portal vein (Edwards *et al*., 1996). This effect can be attrributed to the inhibition of the production of 20-hydroxyeicosatetrianoic acid (20-HETE), an endogenously formed inhibitor of BK_{Ca} channels (Zou *et al*., 1996). The effects of 17-ODYA on the endothelium-dependent hyperpolarization to acetylcholine observed in the guinea-pig internal carotid artery could be explained in part by the hyperpolarization produced by the compound under resting conditions, as the amplitude of the hyperpolarization is negatively correlated to the absolute value of the resting membrane potential. A similar negative correlation has also been observed in the rabbit mesenteric artery (Murphy & Brayden, 1995). The restoration of the endothelium-dependent hyperpolarization (inhibited by 17-ODYA) by iberiotoxin further rules out the involvement of BK_{Ca} channels in the response to EDHF.

Epoxygenase products, incorporated in the endothelial cell lipids, could represent a stored form of EDHF, and could account for the ineffectiveness of cytochrome P_{450} inhibitors and EDHF being a cytochrome P_{450} product since *de novo* synthesis of EDHF would not be required (Mombouli & Vanhoutte, 1997; Weintraub *et al*., 1997). However, 5,6-EpETrE, 8,9-EpETrE, 11,12-EpETrE and 14,15-EpETrE, even at high concentration, did not induce either hyperpolarization or relaxation (whereas acetylcholine was able to induce both in the same conditions), indicating that the involvement of metabolites of arachidonic acid through the cytochrome P_{450} mono-oxygenase pathway in the endothelium-dependent hyperpolarization of the guinea-pig internal carotid artery is most unlikely. In the rat hepatic and mesenteric arteries, most of the studies also concluded that the involvement of the cytochrome P_{450}-derived metabolite of arachidonic acid in the EDHF response was minimal or absent (Zygmunt *et al*., 1996; Fukao *et al*., 1997; Van de Voorde & Vanheel, 1997; Vanheel & Van de Voorde, 1997; in contrast to the study by Chen & Cheung, 1996). The discrepancy between these works in guinea-pig and rat arteries and previous findings suggesting the involvement of cytochrome P_{450} metabolites in endothelium-dependent hyperpolarization and relaxation (i.e. bovine coronary artery, and bioassay system with either human cultured endothelial cells or porcine perfused coronary artery segments: Campbell *et al*., 1996; Popp *et al*., 1996) could be due to a species difference. Likewise, cytochrome P_{450} metabolites can be considered as putative EDHFs in bovine but not in porcine coronary arteries (Graier *et al*., 1996a). In conclusion, these results indicate that, in the guinea-pig internal carotid artery, the involvement of metabolites of arachidonic acid through the cytochrome P_{450} pathway in the endothelium-dependent hyperpolarization induced by acetylcholine is unlikely. Furthermore, the hyperpolarization mediated by EDHF does not involve the activation of BK_{Ca} channels.

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